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A SCANNING ELECTRON MICROSCOPE STUDY OF *ASCIDIA MALACA* EGG (TUNICATE). CHANGES IN THE CELL SURFACE MORPHOLOGY AT FERTILIZATION

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Abstract

Ascidia malaca eggs with and without envelopes were studied using the scanning electron microscope. Follicle cells, chorion, and test cells were examined and compared with those of other ascidian species. No appreciable differences were found. The surface topography of dechorionated eggs differed before and after fertilization.

The pole of the unfertilized egg was indicated by a small smooth region beneath the polar pit. The remaining surface of the egg was undulated. Short microvilli were scattered on the cell membrane except in the area nearest the polar pit. Surface displacements occurred in the fertilized egg, changing its morphological features, at various intervals after sperm penetration. The smooth region expanded shortly after fertilization, and the inferior part of the vegetal hemisphere was corrugated by pronounced undulations. Two new types of microvilli appeared. After the ejection of the first polar body the appearance of the egg surface changed: the animal hemisphere became corrugated, bearing numerous short microvilli, and the vegetal hemisphere showed slight undulations. At the vegetal pole microvilli concentrated to form a protuberance. After the ejection of the second polar body a diffusion of elongated microvilli was observed. The present results indicate that during ooplasmic segregation the movement of the cell membrane components produces changes in the surface topography. These govern the rearrangement of the cytoplasm.

Introduction

Ascidian eggs show characteristic deformations at fertilization and in the interval between the ejection of the first and second polar bodies. Cell surface movements after sperm penetration were studied by Ortolani (1955). Colored chalk granules were bound to the plasma membrane to mark the surface of the unfertilized egg. After fertilization their displacement was observed using the light microscope; the polar pit was used as a landmark. Her observations suggested that the movements of the surface and the modifications in shape were caused by a cortical contraction.

The present SEM study examined the morphology of *Ascidia malaca* eggs with and without envelopes. The dechorionated egg was examined both before and after fertilization to observe the modifications which characterize the surface topography. We followed the distribution and organization of the microvilli at different intervals after sperm penetration.

Previous ultrastructural investigations of *Ascidia malaca* eggs have been performed, but they were primarily concerned with oogenesis (Materazzi and Bondi, 1973; Gianguzza and Dolcemascolo, 1978; 1979), egg morphology (La Spina

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Abbreviations: SEM = Scanning electron microscopy; TEM = Transmission electron microscopy.

D'Anna, 1974), sperm morphology (Villa, 1975; Villa and Tripepi, 1983), and egg-sperm interaction (Villa, 1977). No information exists on the egg plasma membrane.

Transmission and scanning electron microscope studies of egg surface changes and early events in ascidian development used only *Ciona intestinalis* (Sawada and Osanai, 1981). The primary objective of this study was to elucidate the mechanism of ooplasmic segregation. The present results not only extend our knowledge of the ascidian egg envelope but also reveal differences in the egg surface between the animal and vegetal hemispheres, which become more evident after fertilization. During ooplasmic segregation significant changes and apparent transposition of the features of the two halves take place. Modifications in the distribution and shape of the surface microvilli continue to occur until the ejection of the second polar body.

Materials and Methods

Adult specimens of *Ascidia malaca* were collected from the Gulf of Palermo. Male and female gametes were obtained surgically from gonoducts of dissected animals. Eggs removed from the oviducts were washed in Millipore-filtered seawater; some were left intact with egg envelopes, others were transferred to agar-coated Syracuse dishes and dechorionated by hand using steel needles.

The experiments were performed at 22°C by fixing unfertilized eggs and eggs 3, 7, and 30 minutes after insemination.

Transmission electron microscopy

For conventional TEM studies, unfertilized and fertilized (3 min after insemination) intact eggs were fixed with 3% glutaraldehide in 0.1 *M* cacodylate buffer in seawater (pH 7.2) containing 4% sucrose for 30 min at room temperature, and postfixed in 1% osmium tetroxide in the same buffer for 1 h at 4°C. The specimens were dehydrated in an ethanol-propylene oxide series and embedded in Dow epoxy resins (Lockwood, 1964). Sections were stained with saturated uranyl acetate and lead citrate (Venable and Coggeshall, 1965), and examined with a Siemens Elmiskop 1b TEM operating at 80 kV.

Scanning electron microscopy

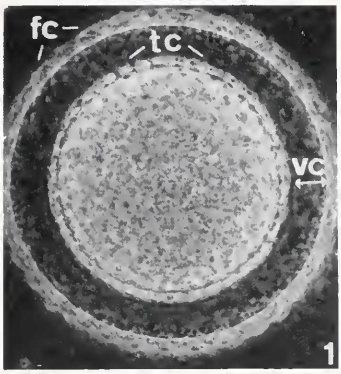
Unfertilized and fertilized intact and hand-dechorionated eggs were fixed as described for TEM, then ethanol-dehydrated, critical-point dried, sputter-coated with gold, and observed with JEOL JSM 15 and ISI DS 130 SEMs. Other intact eggs were fixed and critical-point dried as above, but before they were coated with gold the follicle cell layer and chorion were partially dissected with a fine sharp needle. The dissected specimens were coated and viewed under the SEM as described above.

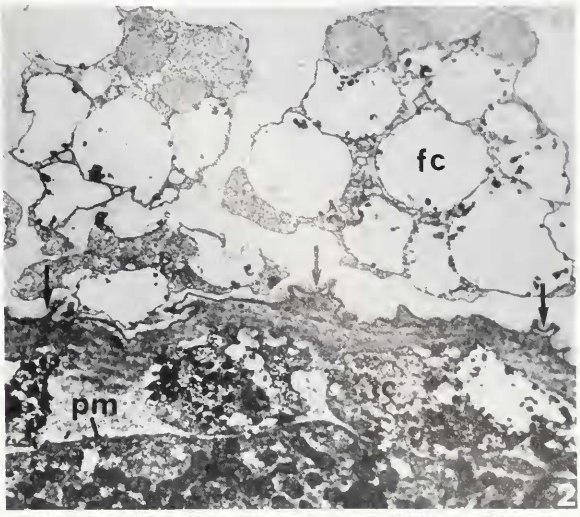
Intact and dechorionated eggs show no evidence of distortion after specimen preparation; however shrinkage is generally observed (about 20%). Shrinkage is greater in intact eggs since the chorion is greatly reduced by SEM preparation.

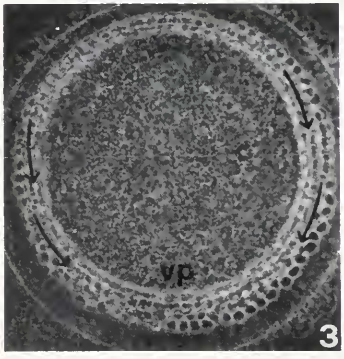
Results

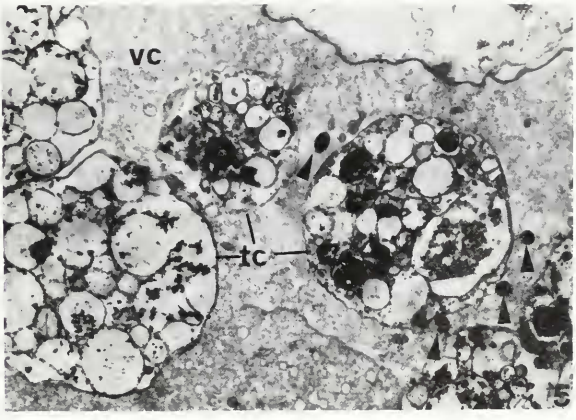
Egg envelope morphology

The *Ascidia malaca* egg envelope consists of an acellular layer—the vitelline coat (*i.e.* chorion)—lying between two cellular layers, the external follicle cells and internal test cells (Figs. 1–3, 5, 6). The follicle cells constitute a single layer of conical-









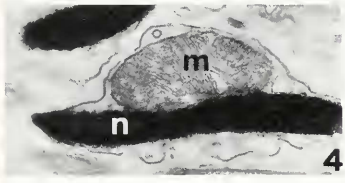


FIGURE 1. Phase contrast micrograph of a living unfertilized *Ascidia malaca* egg. fc = follicle cell; tc = test cell; vc = vitelline coat. 270×.

FIGURE 2. Transmission electron micrograph (TEM) of envelopes of unfertilized egg. Arrows indicate coating of the outer vitelline coat; pm = plasma membrane. 2500×.

FIGURE 3. Phase contrast micrograph of a living fertilized egg; test cells have migrated towards the vegetal hemisphere (arrows). vp = vegetal pole. 290×.

FIGURE 4. TEM of a head of a fully differentiated spermatozoon from the sperm duct. m = mitochondrion; n = nucleus. 10,000×.

FIGURE 5. TEM of envelopes of a fertilized egg; spermatozoa are found in the vitelline coat (arrowheads). 5000×.

shaped, highly vacuolated hexagonally arranged cells; their convex basal region forms indentations in the vitelline coat, which are clearly seen when the latter is cut away (Fig. 11). The follicle cells touch one another only at their basal region, where the plasma membranes of adjacent cells seem to interdigitate, separated by narrow clefts.

The clefts are the only means by which the sperm can reach the subjacent vitelline coat, where the "sperm reaction"—involving swelling, migration, and loss of the mitochondrion—occurs. In fact, many "remnants" of spermatozoa (*i.e.* mitochondrion plus tail), and only a few intact ones, are found mainly in the cleft zone 3 minutes

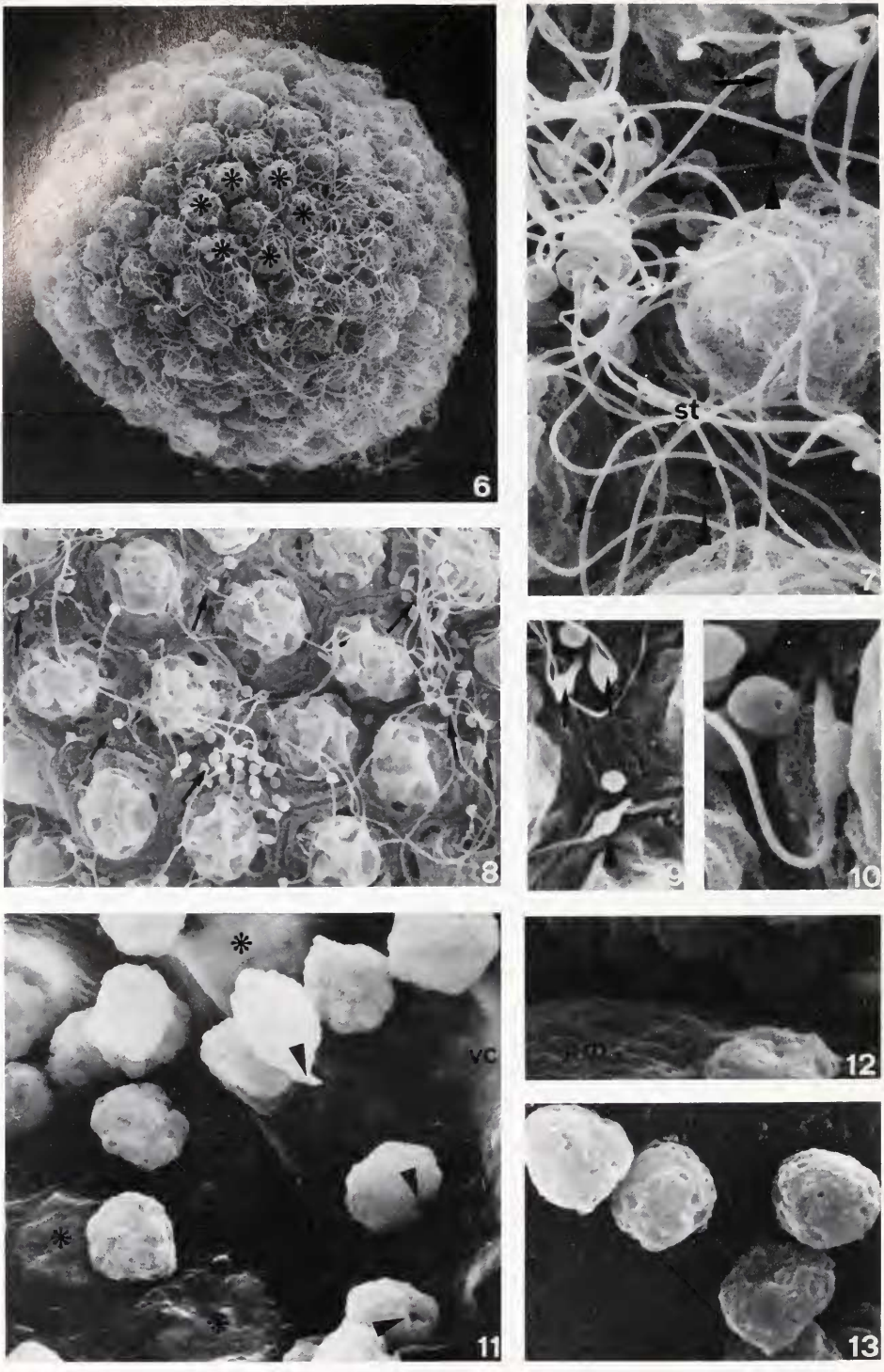


FIGURE 6. Scanning electron micrograph (SEM) of a fertilized egg; follicle cells form a hexagonal pattern (asterisks). 520×.

after fertilization (Figs. 7–10). At high magnification the follicle cells show a reticulated lace-like membrane (Fig. 7).

The chorion shows different morphological features and different thicknesses depending on fixation techniques; however, a thin homogeneous electron-dense outer layer with an organized coating (previously called "chorial membrane," Villa, 1977) and a thicker fibrous inner layer are always observed (Figs. 2, 5). The thickness of the chorion (*ca.* 20 μm *in vivo*) decreases during TEM preparation, and even further during that for SEM.

The chorion encloses the test cells that, in unfertilized eggs, are close to the egg plasma membrane. The test cells form a single but discontinuous layer of roundish or oval, moderately vacuolated cells which move freely within the texture of the chorion.

In fertilized eggs the test cells migrate towards the vegetal hemisphere where they accumulate in layers (Figs. 3, 5).

SEM examination of eggs in which the follicle cell and chorion layers had been partially cut away prior to gold coating revealed that the test cell plasma membranes have either pseudopodia-like extensions or invaginations of corresponding size (Fig. 11); holes can also be observed in the plasma membrane where the numerous microvilli would normally be located (Figs. 12, 13).

Surface morphology

Unfertilized egg. A well-defined polarity is observed: the plasma membrane at the tip of the animal hemisphere is slightly undulated, while the remaining parts are highly undulated with surface folds of random orientation (Fig. 14). The animal pole is marked by a pit, from the bottom of which the first polar body will emerge (Fig. 15). Short microvilli (approximately 0.2 μm in length) are fairly uniformly distributed on the egg surface (Fig. 16) except in the region of the polar pit that is relatively devoid of microvilli.

Eggs 3 min after insemination. The shape of the fertilized egg changes rapidly (Fig. 17): it elongates, becoming pear-shaped with transient bulges, and then rapidly regains its original shape. A marked polarity of the folded areas and two new types of microvilli also characterize this stage. The animal hemisphere and the upper half of the vegetal hemisphere are slightly undulated, while the lower half of the vegetal hemisphere is corrugated (Fig. 19); short microvilli, similar to those of the unfertilized eggs, are scattered over the entire surface except in the polar pit. Moreover, a few

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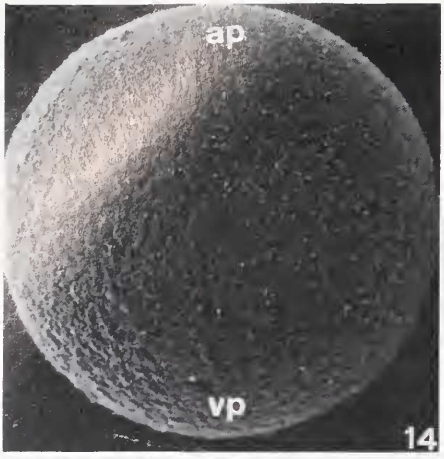
FIGURE 7. High magnification of the follicle cell layer showing clefts between the cells and lace-like reticular membrane. Arrow indicates an intact spermatozoon; arrowheads indicate clefts. st = sperm tail. 3000×.

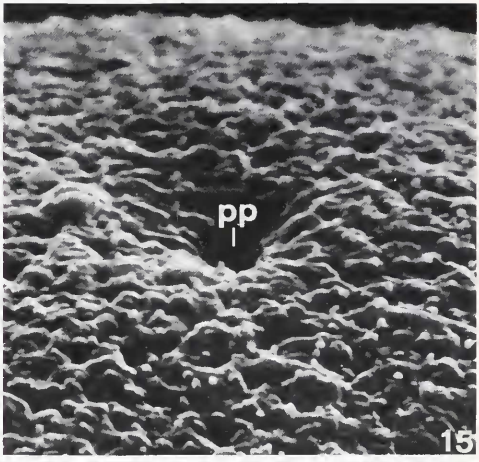
FIGURE 8. SEM of the follicle cell layer of a fertilized egg. Arrows indicate numerous remnants of spermatozoa (*i.e.*, tail + mitochondrion) in the clefts between adjacent follicle cells. 1000×.

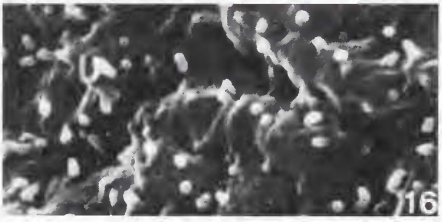
FIGURE 9. SEM of the cleft zone showing intact sperm (arrowhead) and two spermatozoa beginning the "sperm reaction" (arrows). 2000×.

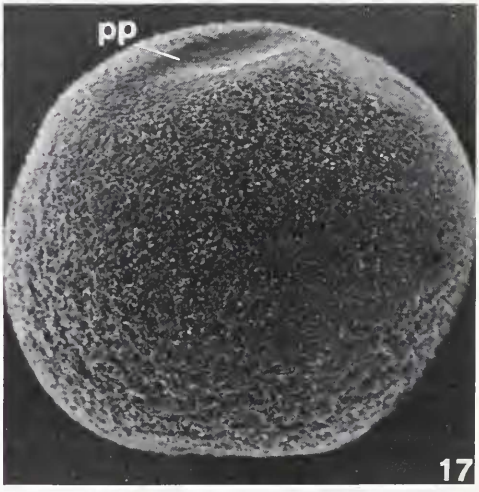
FIGURE 10. High magnification of the cleft zone showing mitochondria left out and a still intact spermatozoon. 4500×.

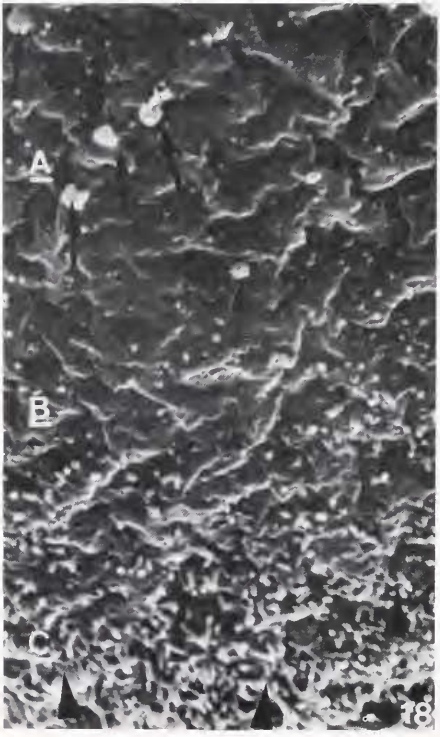
FIGURES 11–13. SEM of unfertilized eggs in which follicle cells and vitelline coat were partially cut away. Figure 11: View of the underside of the vitelline coat showing indentations of the follicle cells (asterisks); numerous test cells with pseudopodia-like extensions or with invaginations (arrowheads) are left on the vitelline coat during dissection. 2710×. Figures 12, 13: View of the egg plasma membrane, on which some test cells rest, showing numerous pore-like openings corresponding to the base of the microvilli. Figure 12, 2050×. Figure 13, 3150×.

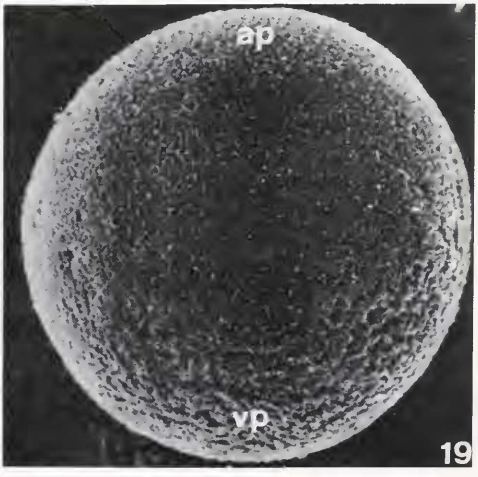












FIGURES 14–16. SEM of unfertilized eggs showing polarity (Fig. 14), smooth and slightly microvillated surface of the egg near the polar pit (Fig. 15), and high magnification of microvilli of highly undulated vegetal area (Fig. 16). ap = animal pole; pp = polar pit. Figure 14, 700×. Figure 15, 5460×. Figure 16, 7600×.

large stumpy microvilli (approximately 0.8 μm in length) are randomly scattered in the equatorial area, and numerous slender microvilli (0.5 μm by 0.1 μm) can be observed very densely arranged in the lower half of the vegetal hemisphere (Fig. 18).

Eggs 7 min after insemination. After the ejection of the first polar body, striking but transient modifications in shape—such as a lobe formation at the vegetal pole (Fig. 21)—occur. An extensive reorganization of the egg surface results in the rearrangement of microvilli and in the apparent transposition of the folded areas from the vegetal to the animal hemisphere. The plasma membrane is highly undulated by pronounced folds in the animal hemisphere (Fig. 20), while in the vegetal hemisphere it is only slightly wrinkled (Fig. 22). The stumpy microvilli of the previous stage are still found in the equatorial area (Fig. 23); the slender microvilli also persist in the vegetal half. At the vegetal pole a well-defined circular protuberance covered by a dense clump of short microvilli appears (Figs. 22, 24); spermatozoa are occasionally encountered on the pole or in the surrounding region.

Eggs 30 min after insemination. The egg regains its spherical shape after ejection of the second polar body. The distribution of the folded areas is similar to that of the previous stage (Fig. 25), although the microvilli reorganize; they are again uniformly distributed over the whole surface and appear slightly longer when compared with those of the previous stages (Fig. 27). Moreover the numerous spermatozoa left on the plasma membrane after insemination are now concentrated at the vegetal pole in which the protuberance of clumped microvilli has gradually faded (Figs. 26, 28).

Discussion

This study provides further information on the morphology of the accessory cells, and new data on nude surface topography.

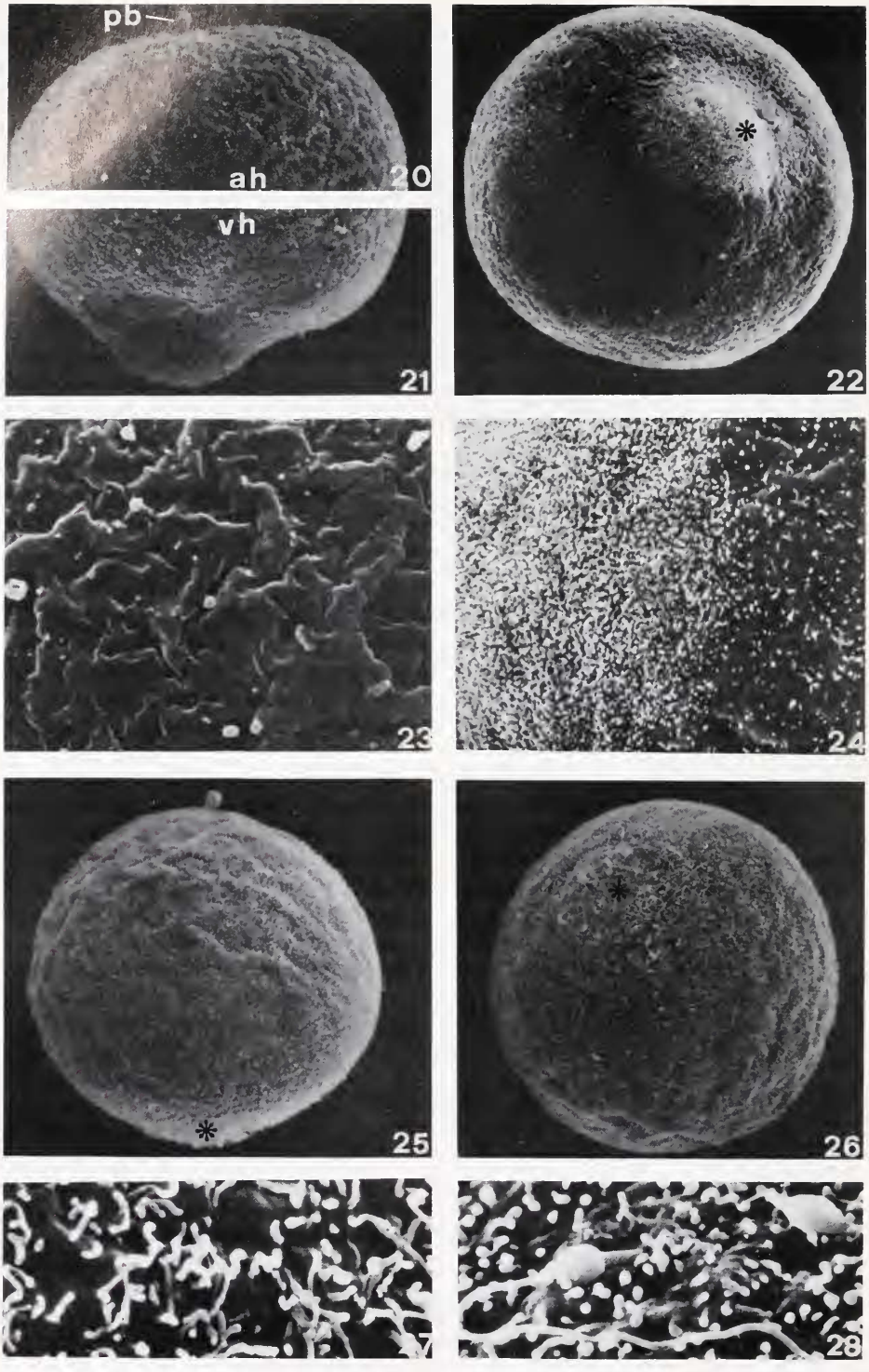
To understand their role in development, egg envelopes of many ascidian species have been subjects of extensive morphological and biochemical studies. However, previous SEM studies of egg envelopes were performed only on a few species including *Ascidiella aspersa* (Mansueto and Villa, 1983), *Ciona intestinalis* (Bates, 1980; De Santis *et al.*, 1980) and *Phallusia mammillata* (Honnegger, 1982, 1986). The general morphological characteristics of *Ascidia malaca* egg envelopes are similar to those of the aforementioned species.

However, our study has detected some peculiarities of the test and follicle cells. The test cell membrane forms pseudopodia and invaginations, which probably reflect dynamic cell-to-cell contact, while the follicle cell membrane shows a reticulated lace-like structure and clefts in the basal region (previously observed only in *Ciona intestinalis,* Bates 1980). The size of these clefts might be involved in the ascidian "sperm reaction" (described by Lambert, 1982): loss of the mitochondrion would reduce the diameter of the spermatozoon to a size allowing easy penetration. Moreover, only intact spermatozoa have been detected on the plasma membrane of dechorionated eggs.

The morphological features of the surface of the dechorionated egg differ greatly before and after fertilization.

A smooth area around the polar pit marks a polarity in the unfertilized egg.

Figures 17–19. SEM of eggs fixed 3 min after fertilization showing shape modification (Fig. 17), different distribution of microvilli in equatorial area: (A) upper vegetal hemisphere (B) and lower vegetal hemisphere (C) (Fig. 18), and marked polarity of the folded areas (Fig. 19). Arrows indicate stumpy microvilli; arrowheads indicate slender microvilli. Figure 17, 700×. Figure 18, 4350×. Figure 19, 700×.



FIGURES 20–24. SEM of eggs fixed 7 min after fertilization showing changes in surface morphology. Figure 20: Supraequatorial area. ah = animal hemisphere; pb = polar body. 620×. Figure 21: Subequatorial area with a lobe formation at the pole. vh = vegetal hemisphere. 620×. Figure 22: View of the vegetal

After sperm penetration and between the ejection of the first polar body and the second maturation division, egg morphology changes drastically. In addition to the known shape deformations which may be caused by a cortical contraction during ooplasmic segregation (Ortolani, 1955; Reverberi, 1971; Sawada and Osanai, 1981), other fine modifications of the surface architecture occur.

In the newly fertilized egg (3 min), the mildly undulated animal area expands over the whole hemisphere as far as the subequatorial zone; the remaining vegetal part is corrugated. This rearrangement corresponds with the displacement of the chalk granules observed with the light microscope: divergent at the animal pole and convergent at the vegetal pole (Ortolani, 1955). These membrane modifications cause the test cells to migrate downwards and accumulate in the lower part of the vegetal region, as described by Conklin (1905).

Changes in the organization and distribution of the microvilli also occur at this time. In addition to the short type found in the unfertilized egg, two new types of microvilli appear: large stumpy microvilli in the equatorial area and slender ones condensed in the vegetal hemisphere, probably where the myoplasm accumulates.

After the ejection of the first polar body (7 min after sperm penetration) an apparent transposition of the features of the two halves is observed. The animal region becomes more undulated and is now densely covered by short microvilli. This occurs almost simultaneously with the disappearance of the polar pit. The vegetal region is less undulated since the egg elongates again, forming a lobe at the pole. This is followed by the ejection of the second polar body. According to the Jeffery and Meier model (1983) the protrusion is formed by a tight contractile ring of actin filaments that seems to push the endoplasm towards the animal pole creating a myoplasmic lobe in the vegetal pole. In the egg which has regained the spherical shape, microvilli concentrate to form a protuberance at the end of the vegetal hemisphere. Supernumerary spermatozoa begin to collect in this region. In fact, the spermatozoa follow the surface movements towards the vegetal pole, as do test cells (Conklin, 1905), chalk granules (Ortolani, 1955), and lectins (Monroy *et al.*, 1973; O'Dell *et al.*, 1974; Ortolani *et al.*, 1977; Zalokar, 1980). The migration of these external components appears to be coordinated with that of the myoplasm with which pigment granules, mitochondria, ribosome-like granules, subcortical granules, and filamentous structures cosegregate (Conklin, 1905; Reverberi, 1956; Berg and Humphreys, 1960; Mancuso, 1964; Sawada and Osanai, 1981; Jeffery and Meier, 1983).

When the egg is mature it is again spherical. Its surface is undulated primarily in the animal region and exhibits an almost homogeneous distribution of elongated microvilli; a small clump of spermatozoa stays at the vegetal pole.

We did not observe development after the fusion of the pronuclei began. We suggest that the elongation and diffusion of the microvilli might constitute a reserve of plasma membrane for the two cell stage. In *Halocynthia roretzi* (Satoh and Deno,

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hemisphere showing the vegetal pole covered by clumped microvilli (asterisk). 620×. Figure 23: Magnification of the equatorial area showing stumpy microvilli. 3300×. Figure 24: Magnification of the clumped microvilli at the vegetal pole. 2550×.

FIGURES 25–28. SEM of eggs fixed 30 min after fertilization. Figure 25: Distribution of folded areas in animal and vegetal hemispheres; the vegetal pole is marked by clumping of supernumerary spermatozoa (asterisk). 580×. Figure 26: View of the vegetal hemisphere showing the polar zone covered by spermatozoa (asterisk). 580×. Figure 27: Magnification of elongated microvilli. 4730×. Figure 28: Magnification of the vegetal polar zone showing intact spermatozoa. 4980×.

1984) the appearance and disappearance of microvilli is associated with cleavage cycles.

Disappearance, concentration, and elongation of the microvilli in different regions of the activated egg could be the expression of a dynamic condition of the plasma membrane. Not only can the microvilli be considered a reserve of membrane, but they could also reflect a reorganization of the cell surface on which cytoplasmic events depend.

Changes in density, distribution, and organization of the microvilli have been observed after fertilization in sea urchin (Eddy and Shapiro, 1976; Schroeder, 1979; Longo, 1986), fish (Iwamatsu, and Keino, 1978), mouse (Nicosia *et al.*, 1978), and amphibian eggs (Monroy and Baccetti, 1975; Charbonneau and Picheral, 1983). Local surface differentiation consisting of special microvilli occur on the polar lobes in the egg of some gastropods (Dohmen and Van der Mey, 1977); it is therefore suggested that a relationship exists between the surface structures and the localization or expression of the morphogenetic factors in the polar lobes.

In ascidian eggs the cell surface elements seem to be connected to the cytoskeleton; the presence in the cortex of a contractile actin-network which produces the force causing cytoplasmic movements has been demonstrated in *Styela, Boltenia,* and *Ciona* eggs (Jeffery and Meier, 1983, 1984; Sawada and Osanai, 1984, 1985). According to Jeffery (1984), ooplasmic segregation includes the movements of cell surface components, cytoskeleton, cytoplasmic organelles, and localized maternal mRNA molecules, which are associated in a cytoplasmic complex. These authors, therefore, proposed that the cortical contraction is the main cause of the polarized ooplasmic movements in the ascidian species.

Several other results from studies of *Ascidia malaca* and *Phallusia mammillata* eggs suggest that a pattern of developmental information is localized in the plasma membrane; an early surface specialization which reflects a cytoplasmic compartmentalization of morphogens may also exist (Monroy *et al.*, 1973; O'Dell *et al.*, 1974; Ortolani *et al.*, 1977; Zolokar, 1980). The lack of external K^+ ions affects cellular activities by acting directly on the membrane of the unfertilized egg; the probable rearrangement of the membrane structures provokes, among other things, a change in the detectability of Con A binding sites (Di Pisa *et al.*, 1982). The role of the plasma membrane during embryonic development has been demonstrated by the differentiation of neural structures and of tissue-specific enzyme obtained through trypsin induction (Ortolani *et al.*, 1979).

Based on these considerations we suggest that in ascidian eggs the cell membrane components and related topographic changes could be the first cause of the ooplamsic movements, which are driven by the contractile actin-network connected to the plasma membrane; therefore, ascidian ooplasmic segregation could depend on surface reorganization.

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LITERATURE CITED

BATES, W. R. 1980. A scanning electron microscope study of the structure and fertilization of an ascidian egg, *Ciona intestinalis*. *Can. J. Zool.* **58:** 1942–1946.

BERG, W. E., AND W. J. HUMPHREYS. 1960. Electron microscopy of four-cell stages of the ascidians *Ciona* and *Styela*. *Dev. Biol.* **2:** 42–60.

CHARBONNEAU, M., AND B. PICHERAL. 1983. Early events in anuran amphibian fertilization: an ultrastructural study of changes occurring in the course of monospermic fertilization and artificial activation. *Dev. Growth Differ.* **25:** 23–37.

CONKLIN, E. G. 1905. The organization and cell-lineage of the ascidian egg. *J. Acad. Natl. Sci. Phil.* **13:** 1–119.

DE SANTIS, R., G. JAMUNNO, AND F. ROSATI. 1980. A study of the chorion and the follicle cells in relation to the sperm-egg interaction in the ascidian, *Ciona intestinalis*. *Dev. Biol.* **74:** 490–499.

DI PISA, G., E. PATRICOLO, AND C. MANSUETO. 1982. Modifications to the egg membrane of *Ascidia malaca* induced by the absence of external K^+ ions. Preliminary data. Cortical response, ooplasmic segregation and cell cycle activation. *Acta Embryol. Morphol. Exper. n.s.* **3:** 85–95.

DOHMEN, M. R., AND J. C. A. VAN DER MEY. 1977. Local surface differentiations at the vegetal pole of the eggs of *Nassarius reticulatus, Buccinum undatum,* and *Crepidula fornicata* (Gastropoda, Prosobranchia). *Dev. Biol.* **61:** 104–113.

EDDY, E. M., AND B. M. SHAPIRO. 1976. Changes in the topography of the sea urchin egg after fertilization. *J. Cell Biol.* **71:** 35–48.

GIANGUZZA, M., AND G. DOLCEMASCOLO. 1978. On the ultrastructure of the follicle cells of *Ascidia malaca* during oogenesis. *Acta Embryol. Exper.* **2:** 197–211.

GIANGUZZA, M., AND G. DOLCEMASCOLO. 1979. On the ultrastructure of the test cells of *Ascidia malaca* during oogenesis. *Acta Embryol. Exper.* **2:** 173–189.

HONNEGGER, T. G. 1982. Effects on fertilization and localized binding of lectins in the ascidian, *Phallusia mammillata*. *Exp. Cell Res.* **138:** 446–451.

HONNEGGER, T. G. 1986. Fertilization in ascidians: studies on the egg envelope, sperm and gamete interactions in *Phallusia mammillata*. *Dev. Biol.* **118:** 118–128.

IWAMATSU, T., AND H. KEINO. 1978. Scanning electron microscopic study on the surface change of eggs of the teleost, *Oryzias latipes,* at the time of fertilization. *Dev. Growth Differ.* **20:** 237–250.

JEFFERY, W. R. 1984. Pattern formation by ooplasmic segregation in ascidian eggs. *Biol. Bull.* **166:** 277–298.

JEFFERY, W. R., AND S. MEIER. 1983. A yellow crescent cytoskeletal domain in ascidian eggs and its role in early development. *Dev. Biol.* **96:** 125–143.

JEFFERY, W. R., AND S. MEIER. 1984. Ooplasmic segregation of the myoplasmic actin network in stratified ascidian eggs. *Wilhelm Roux's Arch. Dev. Biol.* **193:** 257–262.

LAMBERT, C. C. 1982. The ascidian sperm reaction. *Am. Zool.* **22:** 841–849.

LA SPINA D'ANNA, R. 1974. Light and electron microscopic study of unfertilized egg of *Ascidia malaca* (Tunicata). *Acta Embryol. Exper.* **1:** 1–15.

LOCKWOOD, W. R. 1964. A reliable and easily sectioned epoxy embedding medium. *Anat. Rec.* **150:** 129–140.

LONGO, F. J. 1986. Surface changes at fertilization: integration of sea urchin (*Arbacia punctulata*) sperm and oocyte plasma membranes. *Dev. Biol.* **116:** 143–159.

MANCUSO, V. 1964. The distribution of the ooplasmic components in the unfertilized, fertilized and 16-cell stage egg of *Ciona intestinalis*. *Acta Embryol. Morphol. Exper.* **7:** 71–82.

MANSUETO, C., AND L. VILLA. 1983. A light and electron microscope study of the ovular envelopes of *Ascidiella aspersa* (Ascidiacea, Tunicata). *Acta Embryol. Morphol. Exper. n.s.* **4:** 81–91.

MATERAZZI, G., AND A. M. BONDI. 1973. Aspetti ultrastrutturali delle cellule testacee e delle cellule follicolari negli ovociti di *Ascidia malaca*. *Riv. Biol.* **66:** 347–359.

MILLER, R. L. 1975. Chemotaxis of spermatozoa of *Ciona intestinalis*. *Nature* **254:** 244–245.

MILLER, R. L. 1982. Sperm chemotaxis in ascidians. *Am. Zool* **22:** 827–840.

MONROY, A., AND B. BACCETTI. 1975. Morphological changes of the surface of the egg of *Xenopus laevis* in the course of development. *J. Ultrastruct. Res.* **50:** 131–142.

MONROY, A., G. ORTOLANI, D. S. O'DELL, AND G. MILLONIG. 1973. Binding of concanavalin A to the surface of unfertilized and fertilized ascidian eggs. *Nature* **242:** 409–410.

NICOSIA, S. V., D. P. WOLF, AND L. MASTROIANNI JR. 1978. Surface topography of mouse eggs before and after insemination. *Gamete Res.* **1:** 145–155.

O'DELL, D. S., G. ORTOLANI, AND A. MONROY. 1974. Increased binding of radioactive concanavalin A during maturation of *Ascidia* egg. *Exp. Cell Res.* **83:** 408–411.

ORTOLANI, G. 1955. I movimenti corticali dell'uovo di Ascidie alla fecondazione. *Riv. Biol.* **47:** 169–179.

ORTOLANI, G., D. S. O'DELL, AND A. MONROY. 1977. Localized binding of *Dolichos* lectin to the early *Ascidia* embryo. *Exp. Cell Res.* **106:** 402–404.

ORTOLANI, G., E. PATRICOLO, AND C. MANSUETO. 1979. Trypsin-induced cell surface changes in ascidian embryonic cells. Regulation of differentiation of a tissue specific protein. *Exp. Cell Res.* **122:** 137–147.

REVERBERI, G. 1956. The mitochondrial pattern in the development of the ascidian egg. *Experientia* **12:** 55–60.

REVERBERI, G. 1971. Ascidians. Pp. 507–550 in *Experimental Embryology of Marine and Freshwater Invertebrates,* G. Reverberi, ed., Elsevier North-Holland, New York.

SATOH, N., AND T. DENO. 1984. Periodic appearance and disappearance of microvilli associated with cleavage cycles in the egg of the ascidian, *Halocynthia roretzi. Dev. Biol.* **102:** 488–492.

SAWADA, T., AND K. OSANAI. 1981. The cortical contraction related to the ooplasmic segregation in *Ciona intestinalis* eggs. *Wilhelm Roux's Arch. Dev. Biol.* **190:** 208–214.

SAWADA, T., AND K. OSANAI. 1985. Distribution of actin filaments in fertilized egg of the ascidian *Ciona intestinalis. Dev. Biol.* **111:** 260–265.

SCHROEDER, T. E. 1979. Surface area change at fertilization: resorption of the mosaic membrane. *Dev. Biol.* **70:** 306–326.

VENABLE, J. H., AND R. COGGESHALL. 1965. A simplified lead citrate stain for use in electron microscopy. *J. Cell Biol.* **25:** 407–408.

VILLA, L. 1975. An ultrastructural investigation of normal and irradiated spermatozoa of a tunicate, *Ascidia malaca. Boll. Zool.* **42:** 95–98.

VILLA, L. 1977. An ultrastructural investigation on preliminaries in fertilization of *Ascidia malaca* (Urochordata). *Acta Embryol. Exper.* **2:** 179–193.

VILLA, L., AND S. TRIPEPI. 1983. An electron microscope study of spermatogenesis and spermatozoa of *Ascidia malaca, Ascidiella aspersa* and *Phallusia mamillata* (Ascidiacea, Tunicata). *Acta Embryol. Morphol. Exp. n.s.* **4:** 157–168.

ZALOKAR, M. 1980. Activation of ascidian eggs with lectins. *Dev. Biol.* **79:** 232–237.